Optimum Designs for Non-Linear Mixed Effects Models in the Presence of Covariates

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SUMMARY: In this paper we present a new method for optimizing designs of experiments for non-linear mixed effects models, where a categorical factor with covariate information is a design variable combined with another design factor. The work is motivated by the need to efficiently design preclinical experiments in enzyme kinetics for a

set of Human Liver Microsomes. However, the results are general and can be applied to other experimental situations where the variation in the response due to a categorical factor can be partially accounted for by a covariate. The covariate included in the model explains some systematic variability in a random model parameter. This approach allows better understanding of the population variation as well as estimation of the model parameters with higher precision.

Key words: Design of experiments; Enzyme kinetics; Fisher Information; Planning experiments; Random effects model.

1. Introduction

Nonlinear mixed effects models have been extensively used in various applications, in particular in evaluation of the population pharmacokinetic and pharmacodynamic parameters in drug development studies, see for example Chapter 5 of Fitzmaurice et al. (2009). It has been shown that design optimization in the mixed models setting can reduce the number of observations per individual and at the same time provide good predictions of the individual responses as well as good estimates of the population parameters including their variances. This is particularly important in studies in which many observations per individual are not feasible, for example when the patients are small children. Further improvements are possible by including covariates which are partially responsible for the inter-individual variability of the observed responses.

There has been some work done on covariate selection in non-linear mixed effects model building. Wu and Wu (2002) consider such a method in application to HIV data (viral decay rate), which are usually very sparse for individuals, although the number of individuals and the inter-individual variation may be large. There is also a large number of covariates available but only some of them are related to the base model parameters and account for inter-individual variation in the measured viral decay rate. The authors compared various methods of choosing a model given the data when there are missing covariate values. There is however an important open question of how to choose the experimental variables so that the observed responses (data) are most informative. The main design variable in this instance is the time of measuring decay rate, but then the question is which individuals, that is which covariate values, will give best information for efficient model building and evaluation.

Ding and Wu (2001) added an indicator variable as a covariate in their mixed effects model of viral decay for antiviral drugs in HIV to represent a treatment. The model parameters depend on the covariate. They compared type I errors for various tests for the hypothesis of

equality of the treatment effects. In Wu and Ding (2002) they examined the effect of various sampling times on the power for identifying a treatment difference. Retout et al. (2007) showed the efficiency of D-optimum designs for the Wald test of such differences. However, they did not consider the choice of covariate values in the design optimization.

Denti et al. (2010) showed that relating the model parameters to a selection of covariates can decrease significantly the inter-subject variability due to random individual effects. A part of the population variability can be explained by the covariates. They also pointed out that this can lead to savings in the numbers of individual observations and so to increasing the efficiency of the experiments. They also noted the potential of including covariate information at the design stage. We are not aware, however, that this has been done in the kind of experimental set up considered in this paper.

Interest in the optimum design of experiments for mixed effects models has increased over recent decades. Various software packages, such as PFIM, PopED, PopDes or POPT, which provide optimum designs for blood sampling times in pharmacokinetic and pharmacodynamic models are now available and a comparison of the software is presented in Nyberg et al. (2014). Although the models allow for covariates, the design domain defined there does not allow for direct application of the software to our problem, since we do not have a free choice of covariate values. In our optimisation problem we choose the optimum replication of each of a set of experimental units which carry specific values of the covariates.

Our work was initially motivated by applications in preclinical studies of evaluating potential drug-drug interactions. The *in-vitro* experiments are done in Human Liver Microsomes (HLMs) as it is in the liver that most of the enzymes responsible for metabolism occur. These are Cytochrome P450 enzymes (CYPs) and they can be partly responsible for the inter-HLM (inter-subject) variability in respect of the drug metabolism, cf. Hasler et al. (1999).

Belle et al. (2000) showed that population analysis of sparse data can reduce coefficients

of variation of some parameters in enzyme kinetic models. In experiments with HLMs they expressed the inter-HLM variability in terms of the parameter V_{max} of a two-enzyme kinetic model. They also found that the variability in this parameter was related to activity of CYP1A2. Including the activity as a covariate into the model reduced the coefficient of variation (CV) of the parameter estimator from 70% to 39% and it also reduced slightly the error variance estimator's CV (intra-HLM variability). Belle et al. (2000) do not however consider optimum planning of the experiment.

In this study we investigated CYP2D6, which is a member of the cytochrome P450 superfamily of enzymes, which are responsible for the catalysis of many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by phenobarbital. The enzyme is known to metabolize some xenobiotics, such as the anti-cancer drugs cyclophosphamide and ifosphamide. However, in the context of the data presented herein we investigated the metabolism by CYP2D6 of a selective prototypical substrate bupropion.

In this paper we present a method of planning experiments where covariates are treated as design variables whose values are chosen, from the set of values in the available subjects, by the experimenter in combination with other treatment factors. In Section 2 we present notation for a general model and a population design, as well as a design optimality criterion. We also derive the model approximation and the information matrix form. Section 3 is devoted to the application in enzyme kinetics, where we briefly present the numerical optimization algorithm. We also show how the criterion of optimality can be extended to allow for a model transformation in case the residuals do not follow the model assumptions and we present the results. Finally we comment on our findings and in Section 4 we give brief conclusions.

2. Theory and methods

2.1 *Modelling*

We denote by $\boldsymbol{x}$ a vector of levels of a treatment factor, continuous or discrete, although in regression models they are usually continuous, such as concentration of a drug injected into blood plasma, time of taking measurements or temperature at which a chemical reaction is run. We also assume that the population under investigation is diverse in its nature and the diversity can be partially explained by some concomitant variables (covariates) of the population members. These, for example, could be the size of a tumor, its grade and the number of affected lymph nodes of cancer patients taking part in a clinical trial or enzyme activity in drug metabolism in preclinical studies. We denote by $\boldsymbol{z}$ a vector of such covariates. If the purpose of the experiment is to estimate and make inferences on some treatment parameters, then the following question arises: are some values of the covariates more informative than others and, if so, what combinations of individuals with the values of the treatment factors should be used? In Section 3 we show that we can improve the efficiency of estimation of Michaelis-Menten model parameters by an optimum selection of liver tissue preparations characterized by enzymes' activities combined with levels of concentration of the drug under investigation.

We denote by I_s a set of available elements of the population $\mathcal{S}$, that is, $I_s = \{1, 2, \ldots, s\}$ and by $I_{(n)}$ any subset of I_s of size n, that is $I_{(n)} = \{(1), (2), \ldots, (n)\}$, where the round brackets are used to index the elements in $I_{(n)}$. For example, I_s could be all potential HLMs which could be included in an experiment and $I_{(n)}$ a set of HLMs actually selected for the experiment. We will call the elements of population $\mathcal{S}$ *subjects*. Furthermore, let $\boldsymbol{x} \in \mathcal{X} \subset \mathbb{R}^t$ and let $\boldsymbol{z} \in \mathcal{Z} \subset \mathbb{R}^q$, where t and q are some natural numbers. In general terms, we can write a response model for the j-th value of $\boldsymbol{x}$, the (k)-th subject and the i-th replication as

$$y_{ij(k)} = \eta\big(\boldsymbol{x}_{j(k)}, \boldsymbol{\beta}_{(k)}\big) + \varepsilon_{ij(k)}, \quad i = 1, \ldots, r_{j(k)},\ j = 1, \ldots, n_{(k)},\ (k) \in I_{(n)}, \tag{1}$$

where η is a model function relating the response to the runs of the experiment. In most applications relevant to the work presented in this paper it is a non-linear function with respect to both the explanatory variables and the parameters. Furthermore, $\boldsymbol{\beta}_{(k)}$ denotes a p-dimensional vector of functions of the covariates $\boldsymbol{z}_{(k)}$ associated with the (k)-th subject, unknown constant parameters $\boldsymbol{\beta} \in \mathbb{R}^{p_1}$ and a p_2-dimensional vector of random effects $\boldsymbol{b}_{(k)}$, that is, $\boldsymbol{\beta}_{(k)}^{\mathrm{T}} = \big(g_{(k)1}(\boldsymbol{\beta}, \boldsymbol{b}_{(k)}, \boldsymbol{z}_{(k)}), \ldots, g_{(k)p}(\boldsymbol{\beta}, \boldsymbol{b}_{(k)}, \boldsymbol{z}_{(k)})\big)$, where functions g can be linear or non-linear with respect to both the parameters and the covariates. The total number of observations is $N = \sum_{(k)\in I_{(n)}} \sum_{j=1}^{n_{(k)}} r_{j(k)}$. We assume that the random effects $\boldsymbol{b}_{(k)}$ and the random errors $\boldsymbol{\varepsilon}_{(k)}$ are independent and that

$$\boldsymbol{b}_{(k)} \sim \mathcal{N}_{p_2}(\boldsymbol{0}, \boldsymbol{\Sigma}), \quad \boldsymbol{\varepsilon}_{(k)} \sim \mathcal{N}_{m_{(k)}}(\boldsymbol{0}, \sigma_\varepsilon^2 \boldsymbol{I}), \quad \text{for all} \quad (k) \in I_{(n)}, \tag{2}$$

where $\boldsymbol{\varepsilon}_{(k)}$ denotes the vector of random errors for subject (k) and $m_{(k)} = \sum_{j=1}^{n_k} r_{j(k)}$. We denote by $\boldsymbol{\gamma}$ a vector of all the model parameters of interest, that is

$$\boldsymbol{\gamma} = (\boldsymbol{\beta}^{\mathrm{T}}, \boldsymbol{\sigma}^{\mathrm{T}})^{\mathrm{T}} \in \mathbb{R}^{p_1 + \frac{p_2(p_2-1)}{2}} \times \mathbb{R}_+^{p_2+1}, \tag{3}$$

where $\boldsymbol{\beta}^{\mathrm{T}} = (\beta_1, \ldots, \beta_{p_1})$ and $\boldsymbol{\sigma}$ is the $p_3 = \{p_2 + p_2(p_2-1)/2 + 1\}$-dimensional vector of the variances and covariances of the random effects vector $\boldsymbol{b}$ (elements of matrix $\boldsymbol{\Sigma}$) and the error variance, that is $\boldsymbol{\sigma}^{\mathrm{T}} = (\sigma_1^2, \ldots, \sigma_{p_2}^2, \sigma_{12}, \ldots, \sigma_{p_2-1,p_2}, \sigma_\varepsilon^2)$.

2.2 *Design and criterion of optimality*

Each subject $(k) \in I_{(n)}$ is characterized by some covariates $\boldsymbol{z}_{(k)}$. For an efficient experiment we need to choose subjects, which implies the relevant levels of the covariates, paired with values of the vector $\boldsymbol{x}$. In this paper we assume that for a given level (k) the same covariates are used and we assume that their values do not change with the changes in $\boldsymbol{x}$. Then, the experimental design for subject (k) can be written in the following way:

$$\xi_{(k)} = \left\{ \begin{array}{ccc} \boldsymbol{x}_{1(k)} & \ldots & \boldsymbol{x}_{n_{(k)}(k)} \\ r_{1(k)} & \ldots & r_{n_{(k)}(k)} \end{array} \; ; \; \boldsymbol{z}_{(k)} \right\}, \quad (k) \in I_{(n)},\ \boldsymbol{x}_{j(k)} \in \mathcal{X},\ \boldsymbol{z}_{(k)} \in \mathbb{R}^{q_{(k)}},\ r_{j(k)} \in \mathbb{N}.$$

The replications $r_{j(k)}$ of the support points $(\boldsymbol{z}_{(k)},\ \boldsymbol{x}_{j(k)})$ are natural numbers, that is we consider exact designs. The experiment is performed over a subset $I_{(n)}$ of the available subjects of population $\mathcal{S}$ and the design for the subset is denoted by $\zeta = \left\{\xi_{(1)}, \ldots, \xi_{(n)}\right\}$. We call ζ a *population design*, where subjects (k) and values of the explanatory variables $\boldsymbol{x}$ are the *design variables*. Each subject chosen for the experiment has its individual plan of the experiment (*individual design*) $\xi_{(k)}$.

We are interested in efficient estimation of the model parameters $\boldsymbol{\gamma}$ as defined in (3) and we choose the D-criterion for finding optimum designs. We have q covariates available, but in fact we may be interested in a subset of the covariates only.

We denote the information matrix corresponding to model (1) by $\boldsymbol{M}(\zeta, \boldsymbol{\gamma})$. Then the criterion of optimality can be written as $\psi_{\boldsymbol{\gamma}}(\zeta, \boldsymbol{\gamma}) = \log\det \boldsymbol{M}(\zeta, \boldsymbol{\gamma})$. By definition, the information matrix for $\boldsymbol{\gamma}$ is equal to

$$\boldsymbol{M} = -\mathrm{E}\begin{pmatrix} \frac{\partial^2 \ell}{\partial \boldsymbol{\beta} \partial \boldsymbol{\beta}^{\mathrm{T}}} & \frac{\partial^2 \ell}{\partial \boldsymbol{\beta} \partial \boldsymbol{\sigma}^{\mathrm{T}}} \\ \left(\frac{\partial^2 \ell}{\partial \boldsymbol{\beta} \partial \boldsymbol{\sigma}^{\mathrm{T}}}\right)^{\mathrm{T}} & \frac{\partial^2 \ell}{\partial \boldsymbol{\sigma} \partial \boldsymbol{\sigma}^{\mathrm{T}}} \end{pmatrix}, \tag{4}$$

where ℓ denotes the log-likelihood function for the parameters given the observations. Here, however, the marginal density function of vector $\boldsymbol{y}$ whose entries are as in (1) does not have a closed form. To approximate the distribution a Taylor series expansion of the model is often applied. The resulting linear combination of random variables gives a normal distribution if the variables are normal.

2.3 *Model approximation*

Lindstrom and Bates (1990) and Gilberg et al. (1999) use the first order approximation of the Taylor expansion of the model function η around the fixed parameters and the random effects at their estimates. They are interested in methods for parameter estimation and they assume that there are data available to calculate the required initial estimates. At the design stage there are no data to hand. Hence, we evaluate the approximation at a prior guess of

the fixed effects and at the assumed expectation of the random effects. In our paper we are using a point prior, denoted by $\boldsymbol{\beta}^0$, and we assume that $\mathrm{E}(\boldsymbol{b}_{(k)}) = \mathbf{0}$. That is, we expand the model for $\boldsymbol{\vartheta} = \left(\boldsymbol{\beta}^{\mathrm{T}}, \boldsymbol{b}_{(k)}^{\mathrm{T}}\right)^{\mathrm{T}}$ about $\boldsymbol{\vartheta}^0 = \left(\boldsymbol{\beta}^{0\mathrm{T}}, \mathrm{E}(\boldsymbol{b}_{(k)})^{\mathrm{T}}\right)^{\mathrm{T}} = \left(\boldsymbol{\beta}^{0\mathrm{T}}, \mathbf{0}^{\mathrm{T}}\right)^{\mathrm{T}}$ and approximate the model by truncating the expansion as follows:

$$\eta_{j(k)} \;\cong\; \eta_{j(k)}\big|_{\boldsymbol{\vartheta}^0} + \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\vartheta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \left(\boldsymbol{\vartheta} - \boldsymbol{\vartheta}^0\right) \;=\; \alpha_{j(k)} + \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\beta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{\beta} + \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{b}_{(k)}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{b}_{(k)},$$

where $\alpha_{j(k)} = \eta_{j(k)}\big|_{\boldsymbol{\vartheta}^0} - \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\beta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{\beta}^0$. Here the derivatives are evaluated at known values $\boldsymbol{\vartheta}^0$ and so are known functions. Furthermore, due to the chain rule, the derivatives can be written as $\frac{\partial \eta_{j(k)}}{\partial \beta_l} = \frac{\partial \eta_{j(k)}}{\partial\, g_{(k)l'}} \frac{\partial g_{(k)l'}}{\partial \beta_l}$, $l' = 1, \ldots, p$, $l = 1, \ldots, p_1$ and $\frac{\partial \eta_{j(k)}}{\partial b_{(k)l}} = \frac{\partial \eta_{j(k)}}{\partial\, g_{(k)l'}} \frac{\partial g_{(k)l'}}{\partial b_{(k)l}}$, $l' = 1, \ldots, p$, $l = 1, \ldots, p_2$. Writing the above in the matrix notation we have

$$\eta_{j(k)} = \alpha_{j(k)} + \boldsymbol{f}_{j(k)}^{\mathrm{T}} \boldsymbol{Z}_{(k)} \boldsymbol{\beta} + \boldsymbol{f}_{j(k)}^{\mathrm{T}} \boldsymbol{H}_{(k)} \boldsymbol{b}_{(k)},$$

where

$$\begin{aligned}
\boldsymbol{f}_{j(k)}^{\mathrm{T}} &= \left(\frac{\partial \eta_{j(k)}}{\partial\, g_{(k)1}}, \ldots, \frac{\partial \eta_{j(k)}}{\partial\, g_{(k)p}}\right)\Big|_{\boldsymbol{\vartheta}^0} \\
\boldsymbol{Z}_{(k)} &= \left(\left\{\frac{\partial g_{(k)l'}}{\partial \beta_l}\right\}_{l'=1,\ldots,p;\, l=1,\ldots,p_1}\right)\Big|_{\boldsymbol{\vartheta}^0}, \\
\boldsymbol{H}_{(k)} &= \left(\left\{\frac{\partial g_{(k)l'}}{\partial b_{(k)l}}\right\}_{l'=1,\ldots,p;\, l=1,\ldots,p_2}\right)\Big|_{\boldsymbol{\vartheta}^0}.
\end{aligned}$$

Then, model (1) including all observations for individual (k) can be written as

$$\boldsymbol{y}_{(k)} \cong \boldsymbol{\alpha}_{(k)} + \boldsymbol{F}_{(k)} \boldsymbol{Z}_{(k)} \boldsymbol{\beta} + \boldsymbol{F}_{(k)} \boldsymbol{H}_{(k)} \boldsymbol{b}_{(k)} + \boldsymbol{\varepsilon}_{(k)}, \tag{5}$$

where $\boldsymbol{\alpha}_{(k)}$ is the $m_{(k)}$-dimensional vector of constants $\alpha_{j(k)}$, each repeated $r_{j(k)}$ times, $\boldsymbol{F}_{(k)}$ is the $(m_{(k)} \times p)$-dimensional matrix whose rows are $\boldsymbol{f}_{j(k)}^{\mathrm{T}}$, each row repeated $r_{j(k)}$ times.

We assume that random vectors $\boldsymbol{b}_{(k)}$ and $\boldsymbol{\varepsilon}_{(k)}$ are independent and have multivariate normal distributions as in (2). Hence, the approximate expectation and the dispersion matrix of vector $\boldsymbol{y}_{(k)}$ are, respectively, $\boldsymbol{\mu}_{(k)} = \mathrm{E}(\boldsymbol{y}_{(k)}) \cong \boldsymbol{\alpha}_{(k)} + \boldsymbol{F}_{(k)} \boldsymbol{Z}_{(k)} \boldsymbol{\beta}$ and $\boldsymbol{V}_{(k)} = \mathrm{Var}(\boldsymbol{y}_{(k)}) \cong \boldsymbol{F}_{(k)} \boldsymbol{H}_{(k)} \boldsymbol{\Sigma} \boldsymbol{H}_{(k)}^{\mathrm{T}} \boldsymbol{F}_{(k)}^{\mathrm{T}} + \sigma_\varepsilon^2 \boldsymbol{I}_{m_{(k)}}$. The distribution of $\boldsymbol{y}_{(k)}$ is approximately multivariate normal, that is, $\boldsymbol{y}_{(k)} \underset{approx}{\sim} \mathcal{N}_{m_{(k)}}(\boldsymbol{\mu}_{(k)}, \boldsymbol{V}_{(k)})$, where $\boldsymbol{\mu}_{(k)}$ depends on the vector of parameters $\boldsymbol{\beta}$, while $\boldsymbol{V}_{(k)}$ depends on the vector of the variances and covariances $\boldsymbol{\sigma}$.

2.4 *Fisher Information Matrix*

The log-likelihood function for the full vector of parameters, given the responses of the (k)-th subject, approximated by (5), is

$$\ell_{(k)} = \ell(\boldsymbol{\beta}, \boldsymbol{\sigma}|\boldsymbol{y}_{(k)}) = const. - \frac{1}{2}\big(\boldsymbol{y}_{(k)} - \boldsymbol{\mu}_{(k)}\big)^{\mathrm{T}} \boldsymbol{V}_{(k)}^{-1} \big(\boldsymbol{y}_{(k)} - \boldsymbol{\mu}_{(k)}\big) - \frac{1}{2}\log\det(\boldsymbol{V}_{(k)}).$$

The Fisher Information Matrix for subject $(k) \in I_{(n)}$ is then block-diagonal, i.e., $\boldsymbol{M}_{(k)} = \mathrm{diag}\{\boldsymbol{B}_{(k)}, \boldsymbol{C}_{(k)}\}$, where

$$\boldsymbol{B}_{(k)} = \frac{\partial \boldsymbol{\mu}_{(k)}^{\mathrm{T}}}{\partial \boldsymbol{\beta}} \boldsymbol{V}_{(k)}^{-1} \left(\frac{\partial \boldsymbol{\mu}_{(k)}^{\mathrm{T}}}{\partial \boldsymbol{\beta}}\right)^{\mathrm{T}} = \boldsymbol{Z}_{(k)}^{\mathrm{T}} \boldsymbol{F}_{(k)}^{\mathrm{T}} \boldsymbol{V}_{(k)}^{-1} \boldsymbol{F}_{(k)} \boldsymbol{Z}_{(k)},$$

$$\boldsymbol{C}_{(k)} = \frac{1}{2}\left\{ trace\left(\boldsymbol{V}_{(k)}^{-1} \frac{\partial \boldsymbol{V}_{(k)}}{\partial \sigma_i} \boldsymbol{V}_{(k)}^{-1} \frac{\partial \boldsymbol{V}_{(k)}}{\partial \sigma_{i'}} \right) \right\}_{i,i'=1,\ldots,p_3}$$

and σ_i are elements of the p_3-dimensional vector $\boldsymbol{\sigma}$. Other methods of model approximation may lead to a full information matrix with all non-zero blocks, as it is in Retout and Mentré (2003) who use Taylor series linear expansion around random effects only. In such a case the mixed derivatives in (4) are not zero.

We assume that the subjects are independent. Hence, the information matrix for the whole design, which we will call the *Population Fisher Information Matrix* and denote by $\boldsymbol{M}$, is the sum of the individual information matrices, $\boldsymbol{M} = \sum_{(k)\in I_{(n)}} \boldsymbol{M}_{(k)}$ and the D-optimality criterion function is

$$\log\det \boldsymbol{M} = \log\det \mathrm{diag}\{\boldsymbol{B}, \boldsymbol{C}\} = \log\det \boldsymbol{B} \det \boldsymbol{C} = \log\det \boldsymbol{B} + \log\det \boldsymbol{C},$$

where $\boldsymbol{B} = \sum_{(k)\in I_{(n)}} \boldsymbol{B}_{(k)}$ and $\boldsymbol{C} = \sum_{(k)\in I_{(n)}} \boldsymbol{C}_{(k)}$.

3. Application

3.1 *Enzyme kinetics model*

In a typical enzyme kinetics reaction enzymes bind to substrates and turn them into products. The binding step is reversible while the catalytic step is irreversible. In chemical notation $S + E \longleftrightarrow ES \rightarrow E + P$, where S, E and P denote substrate, enzyme and product.

The reaction rate is represented by the Michaelis-Menten model $v = \dfrac{V_{\max} x}{K_m + x}$, where x is the concentration of the substrate ($[S]$) and $V_{\max}$ and K_m are the model parameters: $V_{\max}$ denotes the maximum velocity of the enzyme and K_m is the Michaelis-Menten constant; it is the value of x at which half of the maximum velocity $V_{\max}$ is reached.

In our example, I_s is the set of all available HLM preparations. We have $s = 47$ and the substrate concentration x is assumed to belong to the interval $\mathcal{X} = [0.3, 50]$. Typically, there would be several concentration levels and each would be used for measuring the response (the reaction rate) from each HLM. In Figure 1 we present observations for the 47 liver microsomal preparations of such a standard experiment. We will call this design *rich*. The differences among the subjects are clearly seen in the values of parameter $V_{\max}$, the horizontal asymptote of the Michaelis-Menten model.

[Figure 1 about here.]

There are six cytochrome P450 enzymes specific for each HLM, characterized by the enzyme activities (covariates $\boldsymbol{z}$, $q = 6$). Enzyme activity describes the intrinsic ability of an enzyme to convert one molecule into another and it is related to the substrate concentration (Michaelis-Menten Kinetics). We model the response function η as the Michaelis-Menten function, where the parameters may depend on the covariate values of the subjects (HLMs), which is indicated by the index (k), that is,

$$\eta\big(x_{j(k)}, \boldsymbol{\beta}_{(k)}\big) = \frac{V_{\max(k)} x_{j(k)}}{K_{m(k)} + x_{j(k)}}.$$

We observe, based on the data set shown in Figure 1 and the related activities, that the highest correlation of the enzyme activities with the Michaelis-Menten model parameters is that of CYP2D6 with the maximum velocity of the enzyme $V_{\max}$. Enzyme activity is a positive continuous variable. For numerical calculations in the example presented in this work we standardized the natural log of the covariate.

If we include just one covariate (activity of one enzyme) related to $V_{\max(k)}$ only, then vector $\boldsymbol{\beta}_{(k)}$ can be written in matrix form as

$$\boldsymbol{\beta}_{(k)} = \begin{pmatrix} V_{\max(k)} \\ \\ K_m \end{pmatrix} = \begin{pmatrix} g_{(k)1} \\ \\ g_{(k)2} \end{pmatrix} = \begin{pmatrix} e^{\beta_0+\beta_1 z_{(k)}+b_{(k)}} \\ \\ e^{\beta_2} \end{pmatrix}.$$

The dimensions here are $p = \dim(\boldsymbol{\beta}_{(k)}) = 2$, $p_1 = \dim(\boldsymbol{\beta}) = 3$, $p_2 = \dim(\boldsymbol{b}_{(k)}) = 1$ and $p_3 = \dim(\boldsymbol{\sigma}) = 2$. Also, $\boldsymbol{b}_{(k)} \sim \mathcal{N}(0, \sigma_b^2)$ and $\boldsymbol{\gamma}^{\mathrm{T}} = (\boldsymbol{\beta}^{\mathrm{T}}, \boldsymbol{\sigma}^{\mathrm{T}}) = (\beta_0, \beta_1, \beta_2, \sigma_b^2, \sigma_\varepsilon^2)$. Matrices $\boldsymbol{F}_{(k)}$, $\boldsymbol{Z}_{(k)}$ and $\boldsymbol{H}_{(k)}$ are as follows:

$$\boldsymbol{F}_{(k)} = \left\{ \left(\mathbf{1}_{r_{j(k)}} \times \boldsymbol{f}_{j(k)}^{\mathrm{T}} \right) \Big|_{\boldsymbol{\vartheta}^0} \right\}_{j=1,\ldots,n_{(k)},\,(k)\in I_{(n)}},$$

where

$$\boldsymbol{f}_{j(k)}^{\mathrm{T}} = \left(\frac{x_{j(k)}}{K_m + x_{j(k)}},\ -\frac{V_{\max(k)} x_{j(k)}}{(K_m + x_{j(k)})^2} \right) \Big|_{\boldsymbol{\vartheta}^0} = \left(\frac{x_{j(k)}}{e^{\beta_2^0} + x_{j(k)}},\ -\frac{e^{\beta_0^0+\beta_1^0 z_{(k)}} x_{j(k)}}{(e^{\beta_2^0} + x_{j(k)})^2} \right),$$

$$\boldsymbol{Z}_{(k)} = \begin{pmatrix} e^{\beta_0+\beta_1 z_{(k)}+b_{(k)}} & e^{\beta_0+\beta_1 z_{(k)}+b_{(k)}} z_{(k)} & 0 \\ \\ 0 & 0 & e^{\beta_2} \end{pmatrix} \Bigg|_{\boldsymbol{\vartheta}^0} = \begin{pmatrix} e^{\beta_0^0+\beta_1^0 z_{(k)}} & e^{\beta_0^0+\beta_1^0 z_{(k)}} z_{(k)} & 0 \\ \\ 0 & 0 & e^{\beta_2^0} \end{pmatrix},$$

$$\boldsymbol{H}_{(k)} = \begin{pmatrix} e^{\beta_0+\beta_1 z_{(k)}+b_{(k)}} \\ \\ 0 \end{pmatrix} \Bigg|_{\boldsymbol{\vartheta}^0} = \begin{pmatrix} e^{\beta_0^0+\beta_1^0 z_{(k)}} \\ \\ 0 \end{pmatrix}.$$

These applied to $\boldsymbol{M}$ given in Section 2.4 will give the population FIM for our example.

3.2 *Optimal design search algorithm*

The typical method of finding optimal designs for moderate to large run sizes is to seek continuous optimal designs and round them to the run size required. However, in the case considered here, this is not possible since the optimality criterion is not proportional to the run size N. This is a feature of mixed models where we are interested in the variance components. Instead, we search for exact optimal designs under a local design optimality criterion, for different run sizes, using the Fedorov exchange algorithm.

For each run of the experiment, we must choose an HLM, which implies a choice of a covariate value and a substrate concentration. Since the substrate concentrations are on a continuous scale, we reduce the problem by choosing values from a candidate set. In

practice, this candidate set should be spaced out according to what could be expected to be practically different concentrations. In the following illustration, we used a discrete subset of concentrations from the interval [0.3, 50] and the set of 47 HLMs, which were used to obtain the rich design. We start with a candidate set of treatments which is obtained by all possible combinations of selected concentration levels and 47 HLMs.

A randomly (with replacement) selected N treatments from the candidate set is considered as the initial design, which is then updated using the exchange algorithm. To compete with the initial design, a competing design is obtained by interchanging the first treatment of the initial design by a treatment of the candidate design. The initial and competing designs are compared with respect to the design criterion and the competing design is considered as the current best design (*Case I*) if it corresponds to a higher design criterion value than that of the initial design, otherwise, the initial design is considered as the current best design (*Case II*). To compete with the current best design, the new competing design is obtained either by replacing the second treatment (for *Case I*) or the first treatment of the current best design (for *Case II*) with a new treatment of the candidate set. This search procedure is continued while there is a competing design with higher design criterion value than the current best design; otherwise, the current best design is considered as the optimal design. For exact design, there is no guarantee that the exchange algorithm leads to the global optimal design, so in practice it is preferable to repeat the search procedure for a number of different initial designs to obtain the optimal design.

The R-code is available in Appendix C of the on-line supplementary material.

3.3 *Transformation*

The algorithm described above can be run with a standard D-optimality criterion. However, in practical situations relevant to this work it is often the case that the random errors of observations are not normally distributed. If this is a possible scenario, we propose to trans-

form the model and adjust the optimality criterion to take into account the transformation parameter. We apply the transform both sides method to get

$$y_{ij(k)}^{(\lambda)} = \left\{\eta\big(\boldsymbol{x}_{j(k)}, \boldsymbol{\beta}_{(k)}\big)\right\}^{(\lambda)} + \varepsilon_{ij(k)}, \quad i = 1, \ldots, r_{j(k)},\ j = 1, \ldots, n_{(k)},\ (k) \in I_{(n)},$$

where (λ) indicates the Box-Cox transformation function, that is,

$$y^{(\lambda)} = \begin{cases} \frac{y^{\lambda}-1}{\lambda}, & \text{when } \lambda \neq 0; \\ \log y, & \text{when } \lambda = 0. \end{cases}$$

Gilberg et al. (1999) compared estimation results for a Michaelis-Menten mixed effects model which is weighted and/or transformed on both sides by various methods. For their example the transform both sides models worked better than the non-transformed ones.

The transformation requires adjustments in the linearized model and in the information matrix. For a given value of $\lambda \neq 0$, the derivative of $\eta^{(\lambda)}$ with respect to β_l is $\eta^{\lambda-1}\frac{\partial \eta}{\partial \beta_l}$ and similarly for the derivative with respect to $b_{(k)l}$. Hence, for $\lambda \neq 0$ we have

$$\begin{aligned} \eta_{j(k)}^{(\lambda)} &\cong \eta_{j(k)}^{(\lambda)}\big|_{\boldsymbol{\vartheta}^0} + \eta_{j(k)}^{\lambda-1}\big|_{\boldsymbol{\vartheta}^0} \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\vartheta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \left(\boldsymbol{\vartheta} - \boldsymbol{\vartheta}^0\right) \\ &= \widetilde{\alpha}_{j(k)} + \eta_{j(k)}^{\lambda-1}\big|_{\boldsymbol{\vartheta}^0} \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\beta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{\beta} + \eta_{j(k)}^{\lambda-1}\big|_{\boldsymbol{\vartheta}^0} \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{b}_{(k)}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{b}_{(k)}, \end{aligned}$$

where $\widetilde{\alpha}_{j(k)} = \eta_{j(k)}^{(\lambda)}\big|_{\boldsymbol{\vartheta}^0} - \eta_{j(k)}^{\lambda-1}\big|_{\boldsymbol{\vartheta}^0} \left(\frac{\partial \eta_{j(k)}}{\partial \boldsymbol{\beta}}\big|_{\boldsymbol{\vartheta}^0}\right)^{\mathrm{T}} \boldsymbol{\beta}^0$. Let $\widetilde{\boldsymbol{f}}_{j(k)}^{\mathrm{T}} = \eta_{j(k)}^{\lambda-1}\big|_{\boldsymbol{\vartheta}^0} \boldsymbol{f}_{j(k)}^{\mathrm{T}}$. Then, the linearized transformed model including all observations for individual (k) has the same form as (5) with $\widetilde{\boldsymbol{\alpha}}_{(k)}$ replacing $\boldsymbol{\alpha}_{(k)}$ and $\widetilde{\boldsymbol{F}}_{(k)}$ replacing $\boldsymbol{F}_{(k)}$, where $\widetilde{\boldsymbol{\alpha}}_{(k)}$ is the $m_{(k)}$-dimensional vector of constants $\widetilde{\alpha}_{j(k)}$, each repeated $r_{j(k)}$ times, $\widetilde{\boldsymbol{F}}_{(k)}$ is the $(m_{(k)} \times p)$-dimensional matrix whose rows are $\widetilde{\boldsymbol{f}}_{j(k)}^{\mathrm{T}}$, each row repeated $r_{j(k)}$ times. That is,

$$\boldsymbol{y}_{(k)}^{(\lambda)} \cong \widetilde{\boldsymbol{\alpha}}_{(k)} + \widetilde{\boldsymbol{F}}_{(k)}\boldsymbol{Z}_{(k)}\boldsymbol{\beta} + \widetilde{\boldsymbol{F}}_{(k)}\boldsymbol{H}_{(k)}\boldsymbol{b}_{(k)} + \boldsymbol{\varepsilon}_{(k)}.$$

This model should be now used to obtain the information matrix $\widetilde{\boldsymbol{M}}$ which will have the same form as before but with the adjusted derivatives to account for the transformation.

In our example, vector $\widetilde{\boldsymbol{f}}_{j(k)}^{\mathrm{T}}$ takes the form

$$\widetilde{\boldsymbol{f}}_{j(k)}^{\mathrm{T}} = \left(\frac{\left(\eta_{j(k)}^0\right)^{\lambda}}{e^{\beta_0^0+\beta_1^0 z_{(k)}}},\ \frac{-\left(\eta_{j(k)}^0\right)^{\lambda}}{e^{\beta_2^0}+x_{j(k)}}\right), \quad \text{where} \quad \eta_{j(k)}^0 = \frac{e^{\beta_0^0+\beta_1^0 z_{(k)}} x_{j(k)}}{e^{\beta_2^0}+x_{j(k)}}.$$

The transformation parameter λ is usually unknown and has to be estimated. This means that now we have a multiple objective: efficient estimation of both λ and $\boldsymbol{\gamma}$. Since the joint estimation leads to difficulties with computation and interpretation, to find λ stabilizing the random errors we use the simple fixed effects model (Latif and Gilmour, 2015)

$$y_{ij(k)}^{(\lambda)} = \tau_{j(k)} + \delta_{ij(k)}, \quad \delta_{ij(k)} \sim \mathcal{N}(0, \sigma_\delta^2). \tag{6}$$

This can be considered as a simple ANOVA model, with Box-Cox transformation, with the vector $\boldsymbol{\tau}$ of treatment effects $\tau_{j(k)}$. Optimal design for the ANOVA model with Box-Cox transformation was considered by Atkinson and Cook (1997) for several design criteria, including D_s-optimality for estimating the transformation parameter λ, which we adopt here as part of our compound criterion. We denote by $\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)$ the information matrix for all the parameters of model (6), that is for the treatment effects, the unknown variance and the unknown transformation parameter λ. As we are interested in efficient estimation of the transformation parameter we choose the criterion $\psi_\lambda(\zeta, \lambda) = -\log\left[\mathrm{var}(\widehat{\lambda})\right] = -\log\left[\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}\right]$, where $\boldsymbol{c}^{\mathrm{T}} = (0, 0, \ldots, 0, 1)$ has dimension $\sum_{(k)\in I_{(n)}} n_{(k)} + 2$.

The compound criterion of design optimality for efficient estimation of both the transformation parameter λ and the vector of model parameters $\boldsymbol{\gamma}$, is then

$$\begin{aligned}
\psi(\zeta, \boldsymbol{\gamma}, \lambda) = \psi_\lambda(\zeta, \lambda) + \psi_{\boldsymbol{\gamma}}(\zeta, \boldsymbol{\gamma}) &= -\log\left[\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}\right] + \log\det\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\gamma}|\lambda = \lambda^0) \\
&= \log\frac{\det\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\gamma}|\lambda = \lambda^0)}{\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}} = \log\frac{\det\widetilde{\boldsymbol{B}}(\zeta, \boldsymbol{\beta}|\lambda = \lambda^0)\det\widetilde{\boldsymbol{C}}(\zeta, \boldsymbol{\sigma}|\lambda = \lambda^0)}{\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}} \\
&= \log\frac{\det\widetilde{\boldsymbol{B}}(\zeta, \boldsymbol{\beta}|\lambda = \lambda^0)}{\sqrt{\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}}} + \log\frac{\det\widetilde{\boldsymbol{C}}(\zeta, \boldsymbol{\sigma}|\lambda = \lambda^0)}{\sqrt{\boldsymbol{c}^{\mathrm{T}}\widetilde{\boldsymbol{M}}(\zeta, \boldsymbol{\tau}, \sigma_\delta^2, \lambda)^{-1}\boldsymbol{c}}}.
\end{aligned}$$

3.4 *Numerical results*

The numerical results presented here are obtained for the model introduced in Section 3.3. It should be noted that another covariate structure would produce different numerical output. The fit, $\widehat{y}^{(\lambda)}$, of surface $\eta^{(\lambda)}$ as a function of the concentration and of the covariate (activity of enzyme 2D6, standardized) is shown in Figure 2. We can see that as the covariate value

increases so does the asymptote of the response, that is the value of the parameter $V_{\max}$. The covariate explains some of the variability in the response.

[Figure 2 about here.]

For the single covariate, we obtained designs for $N = 50, 100, 150, 200, 250$ and compared them with the design used for the rich data, which had $2N = 846$, being all combinations of 47 HLMs with the 9 concentrations $\{0.3125, 0.625, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, 50.0\}$ each replicated twice. We used estimates of the parameters obtained from the rich design as prior values for finding the optimum designs which are $\widehat{V}_{\max} = \exp\{\widehat{\beta}_0 + \widehat{\beta}_1 z\}, \widehat{\beta}_0 = -3.15, \widehat{\beta}_1 = 0.74, \widehat{K}_m = \exp\{\widehat{\beta}_2\}, \widehat{\beta}_2 = 1.73, \widehat{\sigma}_b = 0.372, \widehat{\sigma}_\varepsilon = 0.059$. For our optimization we used a regular grid of concentrations in the region of $[0.3,\ 50]$ in steps of 0.1 after refining a coarser grid and the same set of HLMs as in the rich design. The points selected by the algorithm for $N = 50, 100, 150, 200, 250$ are shown in Figure 3 together with the rich design points.

[Figure 3 about here.]

Some patterns, with minor variations, are clear. The structure of these optimum designs is very different from the typical set-up used in practice. Only four concentrations are used, apart from the design for $N = 250$ where there is one point chosen at a fifth concentration. The lowest possible concentration is combined with the HLMs with the lowest covariate values. The second and third chosen concentration values, close to the prior value assumed for K_m, are combined with several HLMs with the highest values of the covariate. Finally, the highest possible concentration is used with many HLMs, starting with those at each extreme and working towards the middle as the run size increases.

The marginal optimal designs shown in Figure 4 indicate the number of replications of the support points for the values of N considered. Both concentration and the activity values on the borders of the design region are chosen much more often than the internal points.

[Figure 4 about here.]

In particular, it is interesting to observe two characteristics of the marginal design for the choice of covariate values: first, that all the available values are chosen when $N = 100$ or more, and second that the weight is mostly put on the border values. The former characteristic is related to the fact that the response depends on the covariate z via the parameter $V_{\max}$ and the latter is related to the fact that the response depends linearly on $V_{\max}$ and so the end points of the parameter region will be most informative.

Note that this shows only the covariate value and does not distinguish between different HLMs with the same value, some of which existed in the data set used. The designs are given in full in Tables 1–5 of Appendix B and the original data are given in Appendix A; both appendices are in the on-line supplementary material.

Furthermore, looking at the marginal design for the concentration values, we see that a large weight is put on the biggest concentration value, which gives information on the $V_{\max}$ parameter, which is considered as random. On the other hand, parameter K_m is assumed to be constant and so only some of the covariate values are combined with the concentrations which give information on this parameter.

We use a relative efficiency measure for a population design ζ compared with another design, for example a standard design used in practice. In our case we compare the optimum design $\zeta^\star$ and the rich design ζ^{rich} in a space of population designs Δ, that is

$$Eff = \left\{ \frac{\det \boldsymbol{M}(\zeta^\star, \boldsymbol{\gamma}^0)}{\det \boldsymbol{M}(\zeta^{rich}, \boldsymbol{\gamma}^0)} \right\}^{\frac{1}{\dim(\boldsymbol{\gamma})}},$$

where both designs are evaluated at some prior parameter values $\boldsymbol{\gamma}^0$.

The relative efficiencies of the optimal designs obtained for various values of N to the rich design are shown in Figure 5. We also calculated the relative efficiency for the optimal design replicated 2, 3, 4 and 5 times.

[Figure 5 about here.]

We can see that two replications of a 250-point optimal design is as good as the rich design (which is a 423-point design replicated twice). We could use 500 runs of the experiment rather than 846 to obtain the same efficiency of estimation if we do the design optimization including a covariate which explains a significant part of the response variability.

The 50-point design, even when replicated 5 times, is worse than the rich design. This is because it does not carry enough information on the variable parameter $V_{\max}$; not all available covariate values are combined with the largest value of the concentration. On the other hand, the 100-point design replicated five times is almost equally efficient as the 250-point design replicated twice. In fact, in both designs all covariate values are combined with the largest concentration value. Furthermore, if we could use a similar number of runs as in the rich design, then for example, the 200-point design replicated four times would give a higher efficiency, still with slightly fewer runs. Of course, the rich design might have other advantages, especially in allowing for checking the assumed functional form of the model, which are not captured by the optimality criterion. However, this was not a major concern in the application considered here, where the enzyme mechanism is well understood.

The results are based on the linear approximation of the model. For fixed effects models there are tools to check both the intrinsic and parameter curvatures; c.f., Bates and Watts (1980). For such models design efficiency criteria have been suggested, as in Hamilton and Watts (1985). Bogacka and Wright (2004) have compared D-optimality with Hamilton and Watts' Quadratic criterion, which incorporates measures of both curvatures. They also considered a constrained design strategy, where the number of design replications ensures that the curvature is less than some fixed threshold. As is shown in Bates and Watts (1980) the curvatures are reduced by a factor of $1/\sqrt{r}$, where r denotes the replication of the design. It might therefore be better to use an optimum design with a smaller number of points, but replicated more times, such as 10 replications of the 50-point optimum design. However, to

the best of our knowledge, no work has been done regarding the effect of curvature on the robustness of experimental design for nonlinear mixed effects models with covariates treated as design variables. This is an important and interesting problem to consider.

We performed 5000 simulations to examine the precision of parameter estimation of the model using two replications of the 150-, 200-, and 250-point optimum designs, and of the 423-point rich design, as well as 10 replications of the 50-point optimum design. The estimates based on the rich data are used as the true values to simulate the response. The results are reported in Table 1. For each of the estimates, the bias, obtained by subtracting the true value from the sample mean over the simulations, the sample standard deviation over the simulations, and the average, over the simulations, of the estimated standard error are reported.

[Table 1 about here.]

There is not much difference in the precision of estimation, but the rich design gives slightly smaller bias in estimating the transformation parameter λ. However, the estimated standard error of $\hat{\lambda}$ is closer to the simulated standard deviation for the optimal designs compared with that of the rich design.

The comparison between two replications of the 250-point optimum design and 10 replications of the 50-point optimum design shows that the latter gives smaller bias of all the fixed parameters and of the error variance. However, the bias of the variance σ_b^2 of the random effect is almost twice as big from 10 replicates of the 50-point design as from two replicates of the 250-point design. This may be indicating some properties of such designs in the case of models with parameters being functions of covariates and random effects. In our case $V_{\max}$ is modelled as a linear function of the enzyme activity and an additive random variable. The 250-point design puts substantial weight on the ends of the design region, as seen in Figure 4(e). In particular, large weights on the end-points of the covariate's domain as well as the

use of all covariate values at the end of the concentration's domain will be important for estimation of σ_b^2, a parameter evaluating the population variability due to the covariate.

4. Conclusions

In this paper we present a new method of designing experiments for non-linear mixed effects models where covariates are design variables combined with an explanatory variable or another treatment factor. We give forms of the information matrix and of the D-optimality criterion for such a case and also expand the criterion to allow for transformation of the response in case of non-normal random errors.

The theory is exemplified by data on Human Liver Microsomes with various enzyme activities. Several optimized designs are presented and their properties studied. We observe that substantial savings can be achieved by using such designs. The new designs can be equally efficient using less experimental material than is needed in standard practice or, if a similar experimental effort is allowed, then we can achieve higher efficiency.

Furthermore, using mixed-effects models with covariates we gain information on the population variability and are able to asses the variation of the response due to the covariates. This can be useful for stratification of the population and also for personalizing the treatments.

In this paper we assume that we know which covariate is important for the response and we choose values of this covariate to optimize the design. More work is needed to further develop the methodology to optimally choose among several covariates during the stage of designing experiments.

5. Supplementary Materials

The original data are given in Web Appendix A; Tables 1–5 containing the design points are given in Web Appendix B; the R code can be found in Web Appendix C, all at Biometrics website at the Wiley Online Library.

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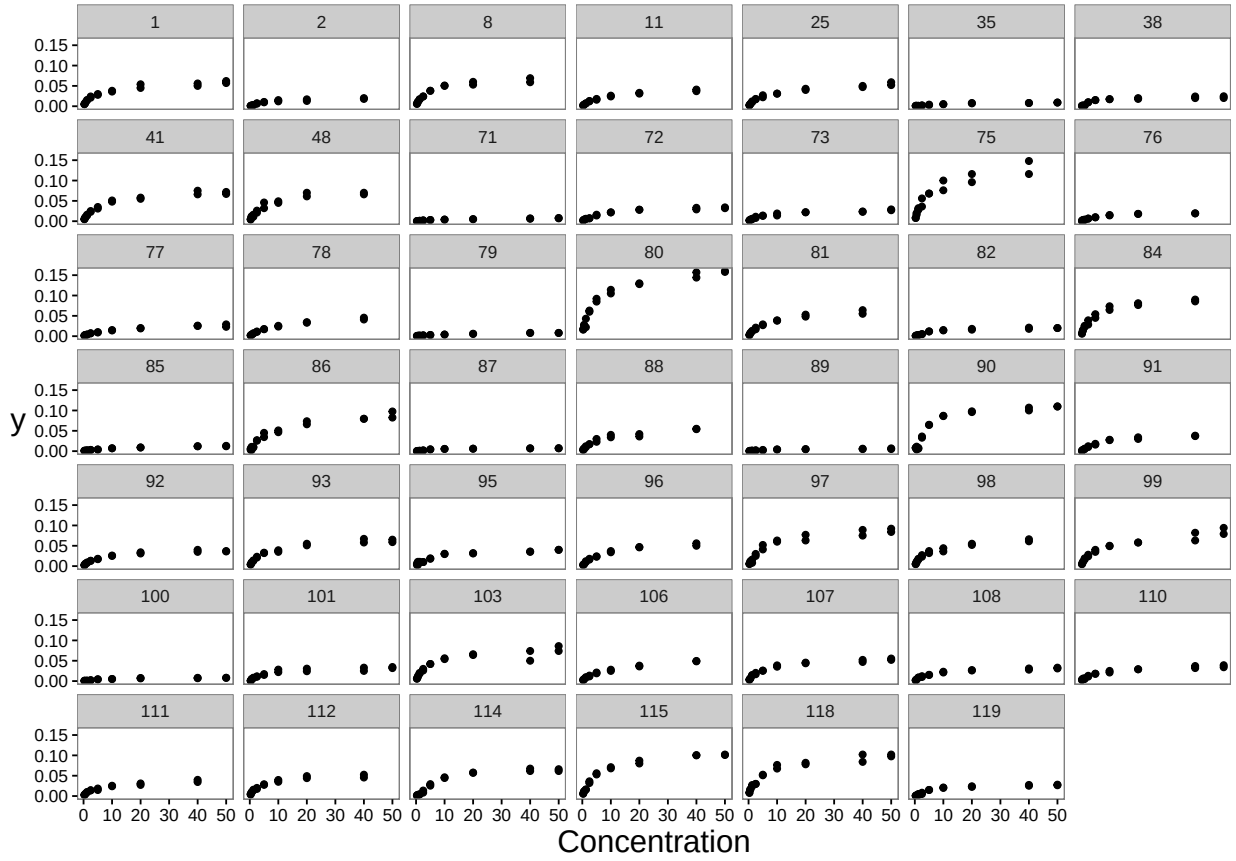


Figure 1. Observations of reaction rates for a substrate in 47 HLM preparations. There are two observations at each of the design points (substrate concentrations).

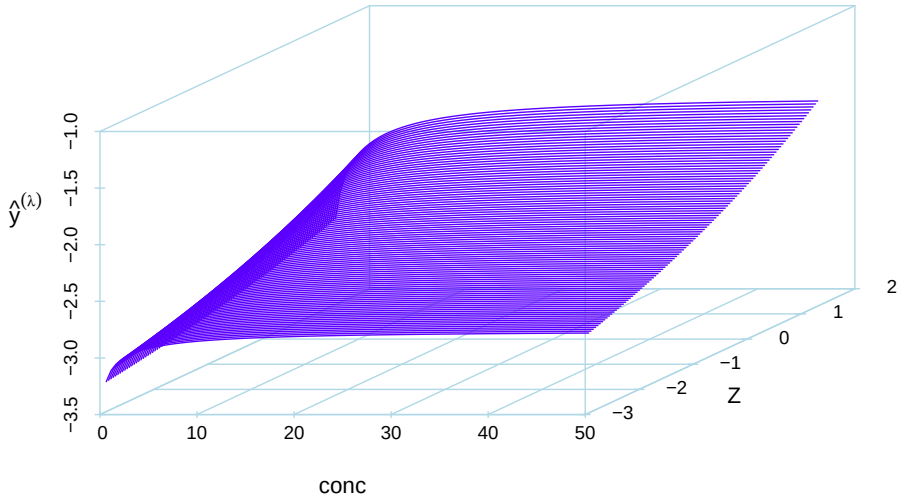
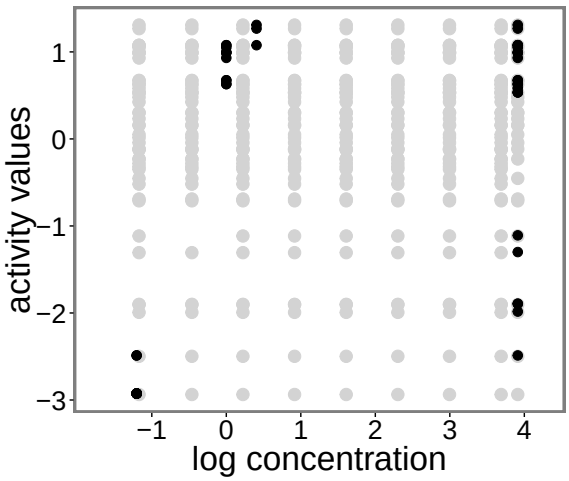
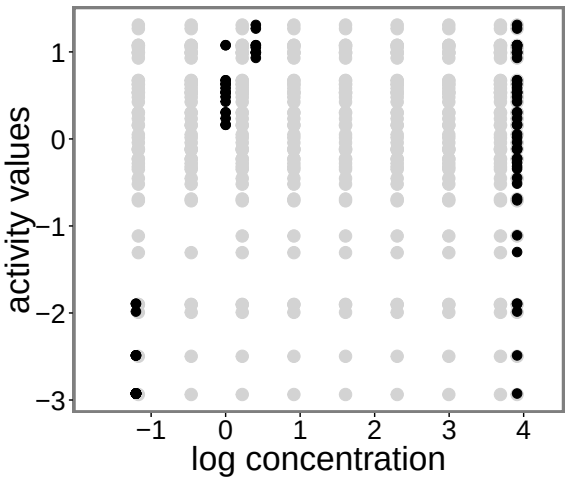


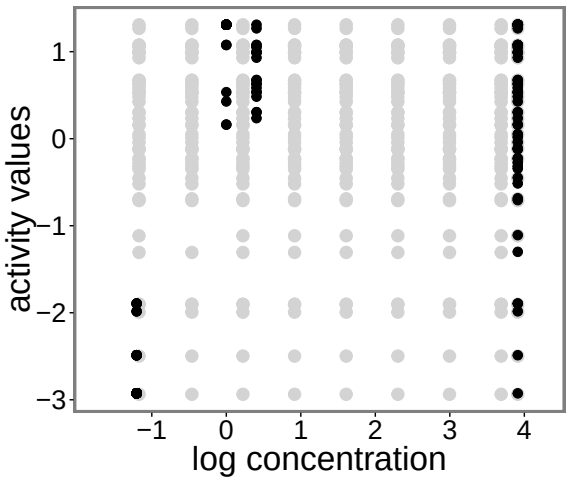
Figure 2. The fit of transformed model surface as a function of the concentration and the normalized log of the covariate (based on the rich data set).



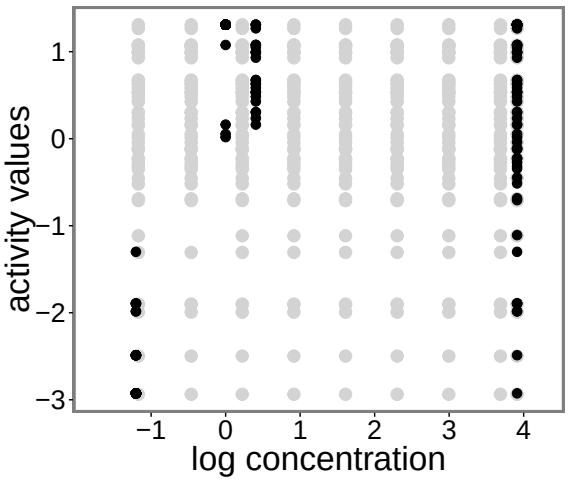
(a) $N = 50$



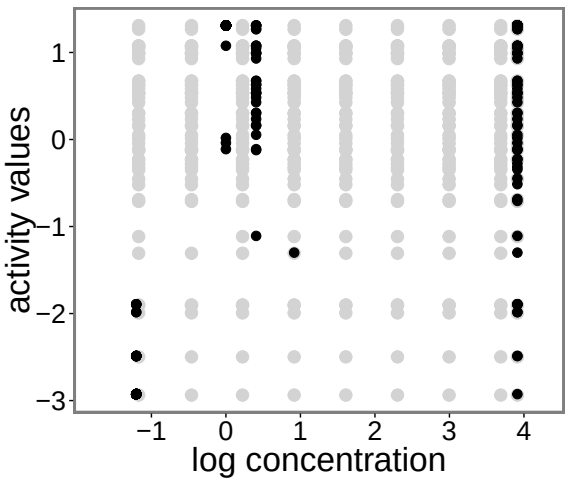
(b) $N = 100$



(c) $N = 150$



(d) $N = 200$



(e) $N = 250$

Figure 3. Optimum concentration levels and activity values obtained for five different values of the total number of observations N (grey points indicate rich design).

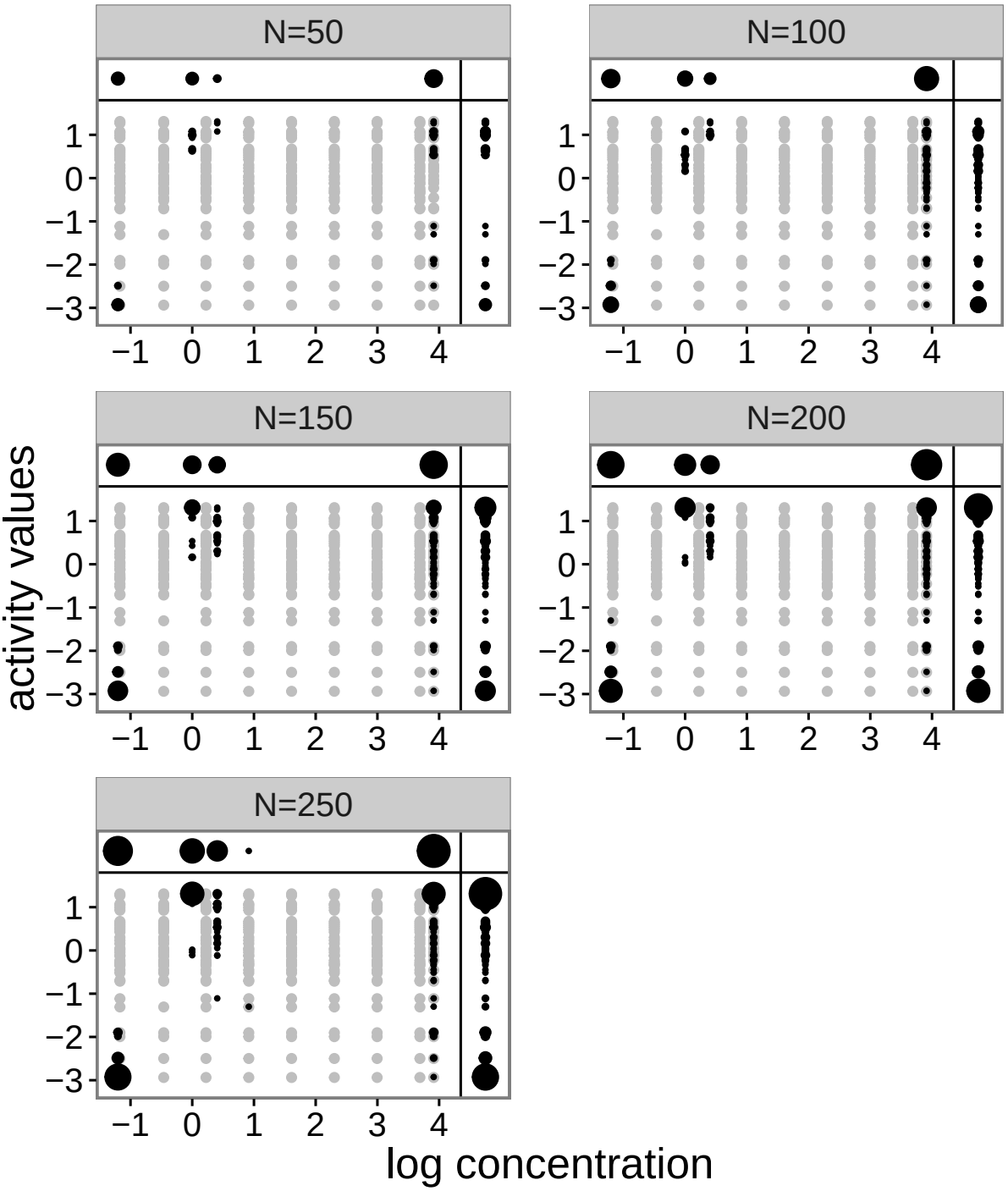


Figure 4. Support points of the optimal designs and the marginal designs (grey dots indicate rich design). The diameter of a dot indicates the number of replications of the support point.

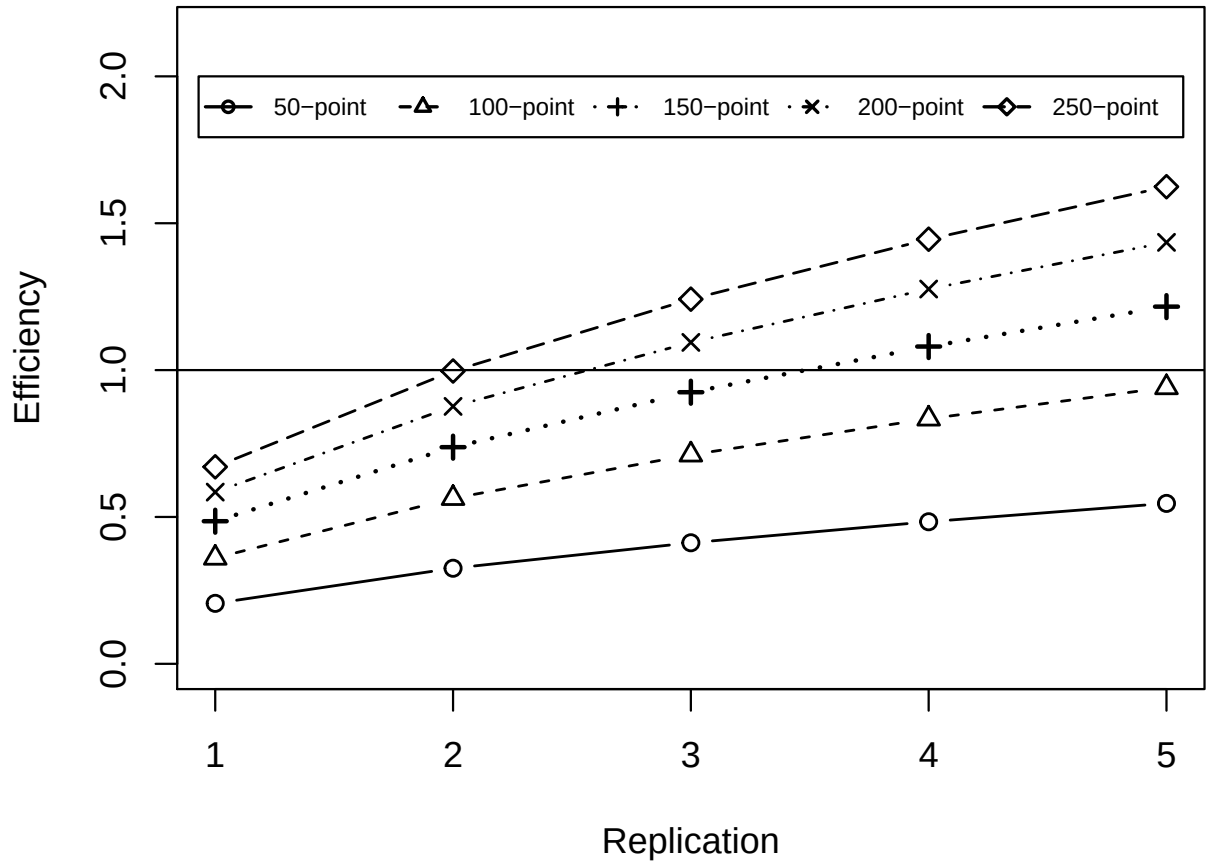


Figure 5. Relative efficiencies of the optimal designs, and of replicates of these designs, with respect to the rich design.

Table 1

Simulation results for comparing two replicates of 150-, 200-, and 250-point optimum designs, 10 replicates of 50-point optimum design and two replicates of 423-point rich design. The results are based on 5,000 simulations and estimates obtained from the rich data are used as true values.

$r \times N$		β_0	β_2	β_1	σ_b	σ	λ
2×150	bias	0.0044	-0.0005	-0.0009	-0.0083	-0.0076	0.0292
	sd	0.0556	0.0320	0.0567	0.0406	0.0057	0.0198
	se	0.0556	0.0312	0.0560			0.0174
	sd/se	1.0006	1.0246	1.0128			1.1383
2×200	bias	0.0043	-0.0001	-0.0011	-0.0100	-0.0054	0.0203
	sd	0.0556	0.0278	0.0570	0.0410	0.0050	0.0171
	se	0.0552	0.0264	0.0554			0.0149
	sd/se	1.0076	1.0534	1.0293			1.1462
2×250	bias	0.0027	-0.0005	-0.0010	-0.0096	-0.0044	0.0163
	sd	0.0559	0.0237	0.0560	0.0405	0.0044	0.0150
	se	0.0550	0.0233	0.0551			0.0133
	sd/se	1.0157	1.0206	1.0152			1.1239
10×50	bias	0.0000	-0.0001	0.0003	-0.0176	-0.0018	0.0074
	sd	0.0746	0.0261	0.0567	0.0530	0.0043	0.0144
	se	0.0727	0.0256	0.0552			0.0142
	sd/se	1.0254	1.0195	1.0263			1.0156
2×423	bias	0.0006	0.0002	-0.0005	-0.0102	0.0009	-0.0016
	sd	0.0551	0.0223	0.0550	0.0388	0.0072	0.0270
	se	0.0543	0.0222	0.0542			0.0193
	sd/se	1.0162	1.0073	1.0145			1.3977
Rich data	est	-3.1547	1.7268	0.7439	0.3730	0.0591	0.2800
	se	0.0556	0.0223	0.0555			0.0192